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September 28, 2005

### REMARKS

Applicants have amended their claims in order to further clarify the definition of various aspects of the present invention. Specifically, previously considered claims 1-5 have been cancelled without prejudice or disclaimer, and new claims 8-11 have been added to the application; In addition, withdrawn claims 6 and 7 have been maintained in the application, subject to the filing of a Divisional application directed to the subject matter thereof.

Claim 8 recites a method for gene expression analysis, including preparing first and second nucleotides each including a targeted gene by using first and second samples, respectively, and introducing recited base sequences which are nonspecific to the base sequence of the targeted gene, the introduced base sequences being positioned relative to the 5' end; subjecting the first and second nucleotides to nucleic acid amplification using various recited primers and probes, the primers being a primer comprising a base sequence specifically hybridizing to the targeted gene and a primer identical to the second base sequence the probes being a first probe comprising a base sequence identical or complementary to the first base sequence, and labeled at one end with a first fluorophore and at another end with a quencher, and a second probe comprising a base sequence identical or complementary to the third base sequence and labeled at one end with a second fluorophore and at another end with a quencher, and thermostable DNA polymerase; digesting the first and second probes; and detecting a fluorescence emitted by the first and second fluorophores released in digesting the first and second probes. Note, for example, Fig. 6 of Applicants' original disclosure, together with the description in connection therewith on pages 12-15 of Applicants' specification. Compare claim 8 with original claim 1.

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New claim 9, dependent on claim 8, further defines how the first and second nucleotides are synthesized; and claim 10, also dependent on claim 8, recites that the first and second nucleotides are cDNA comprising recited base sequences introduced by subjecting mRNA of the targeted gene to reverse transcription using defined primers. Claim 11, dependent on claim 8, recites that the  $T_m$  values of the first and second probes are substantially the same.

In connection with claims 9-11, note, e.g., previously considered claims 2, 3 and 5, respectively. Note also the aforementioned Fig. 6 and the aforementioned description in connection therewith on pages 12-15 of Applicants' specification.

The restriction requirement set forth on pages 2-4 of the Office Action mailed June 28, 2005, is noted. Pursuant to the requirement therein, Applicants affirm their election of the Group I claims (claims 1-5), and note that claims 6 and 7 are withdrawn from further consideration. Claims 6 and 7 are being maintained in the present application subject to the filing of a Divisional application directed to the subject matter thereof.

The rejection of claims 2 and 3 under the second paragraph of 35 USC 112, as being indefinite, set forth on page 5 of the Office Action mailed June 28, 2005, is moot, in light the presently submitted claims. Specifically, note that claims 9 and 10 as submitted herewith recite "a" fourth base sequence; in view thereof, it is respectfully submitted that the indefiniteness rejection is moot.

Applicants respectfully submit that all of the claims presented for consideration by the Examiner on the merits, patentably distinguish over the teachings of the references applied by the Examiner in rejecting claims in the Office Action mailed June 28, 2005, that is, the teachings of the U.S. Patent to Shah, et al.,

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No. 6,165,723, and International (PCT) Publication No. WO 97/42345 (Whitcombe, et al.), under the provisions of 35 USC 102 and 35 USC 103.

It is respectfully submitted that the references as applied by the Examiner would have neither taught nor would have suggested such a method for gene expression analysis as in the present claims, including, inter alia, the preparing of the first nucleotides including a targeted gene and preparing the second nucleotides including this targeted gene, by using first and second samples, respectively, and introducing various base sequences which are nonspecific to the base sequence of the targeted gene, to the targeted gene so that the various base sequences are bound at recited positions relative to the 5' end; and subjecting the first and second nucleotides to nucleic acid amplification using various primers and probes, and thermostable DNA polymerase, with digesting of the first and second probes and detecting a fluorescence emitted by respective fluorophores released in digesting the first and second probes, to thereby assay the amount of the product of the nucleic acid amplification. Note claim 8.

In addition, it is respectfully submitted that the teachings of the applied references would have neither disclosed nor would have suggested such method for gene expression analysis as in the present claims, having features as discussed previously in connection with claim 8, and, additionally, the further definition of synthesizing the first and second nucleotides as recited in claim 9; and/or the further definition of the first and second nucleotides as in claim 10; and/or wherein the  $T_m$  values of the first and second probes are substantially the same, as in claim 11.

According to the present invention, first and second nucleotides are prepared respectively from first and second samples and respectively are subjected to nucleic acid amplification using first and second probes respectively having a base

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sequence identical or complementary to base sequences used in preparing the first and second nucleotides, respectively, with each of the first and second probes being labeled at one end with a respective fluorophore and at another end with a quencher. Such technique as in the present invention enables highly accurate quantitative analysis of expression levels of a targeted gene in two or more samples under substantially the same conditions by real-time polymerase chain reaction (PCR). Note, for example, the sole full paragraph on page 12, the second paragraph on page 15, and the sole paragraph on page 22, of Applicants' specification.

Whitcombe, et al. discloses a method for the detection of diagnostic base sequences in sample nucleic acid, using tailed diagnostic primers having a tag region and a detector region. The method includes contacting a sample under hybridizing conditions and in the presence of appropriate nucleoside triphosphates and an agent for polymerization thereof, with a diagnostic primer for the diagnostic base sequence, the diagnostic base primer having a tail sequence comprising a tag region and a detector region, such that an extension product of the diagnostic primer is synthesized when the corresponding diagnostic base sequence is present in the sample, no extension product being synthesized when the corresponding diagnostic base sequence is not present in the sample and any extension product of the diagnostic primer acting as a template for extension of a further primer which hybridizes to a locus at a distance from the diagnostic base sequence; contacting the sample with a tag primer which selectively hybridizes to the complement of the tag sequence in an extension product of the further primer and is extended; and detecting the presence or absence of the diagnostic base sequence by reference to the detector region in the further primer extension product. Note the paragraph bridging pages 1 and 2 of this patent. Note also page 4, lines 22-27, disclosing use

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of diagnostic and further primers which are genome specific at their 3'-termini but which carry a detector region and common extensions (tags) at their 5'-termini. Note also from page 6, line 24 through page 7, line 1; and page 10, lines 7-9, of Whitcombe, et al.

As can be seen in the foregoing, as well as from a full review of Whitcombe, et al., it is respectfully submitted that this reference does not disclose, nor would have suggested, such method as in the present claims, including the preparation of first and second nucleotides including the targeted gene from first and second samples, together with the other processing as in the present claims, and advantages thereof as discussed in the foregoing; and/or other features of the present invention as discussed previously, and advantages thereof.

Even assuming, arguendo, that the teachings of Shah, et al. were properly combinable with the teachings of Whitcombe, et al., it is respectfully submitted that such combined teachings would have neither disclosed nor would have suggested the presently claimed invention.

Shah, et al. discloses a method for detecting a target nucleic acid fragment directly from a specimen obtained from a patient by in situ hybridization, the method including steps in the listed order of:

- (1) depositing a sample of the specimen onto a slide;
- (2) fixing the sample onto the slide with fixative, the fixative comprising either methanol-acetic acid at a ration of from 99:1 to 80:20, or formalin-acetic acid at a ration of from 99:1 to 80:20;
- (3) contacting the nucleic acids of affixed sample with a probe complex specific for the target nucleic acid fragment, under conditions appropriate for hybridization;

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- (4) rinsing non-hybridized probe complex from the sample and staining the rinsed sample with Evans Blue; and
- (5) visually detecting the hybridized probe complex by microscopy, with the presence of the probe complex being an indication of the presence of the target nucleic acid fragment.

See column 1, lines 38-54. See also column 2, lines 4-8, 29-33 and 53-56. Note further, column 4, lines 58-63; and column 5, lines 43-47.

Even assuming, arguendo, that the teachings of Shah, et al. were properly combinable with the teachings of Whitcombe, et al., such combined teachings would have neither disclosed nor would have suggested the presently claimed subject matter, including, inter alia, the preparation of the first and second nucleotides each including the targeted gene by using first and second samples, respectively, as in claim 8, and subjecting, digesting and detecting as in claim 8; and/or the other features of the present invention as discussed previously, and advantages thereof.

In connection with previously considered claim 4, the Examiner contended that Whitcombe, et al. discloses the use of their invention with the Amplification Refractory Mutation System (ARMS) in a multiplex single tube genotyping assay where multiple probes are utilized in one reaction vessel. Even assuming, arguendo, that the Examiner is correct in this interpretation of Whitcombe, et al., it is respectfully submitted that Whitcombe, et al. would have neither taught nor would have suggested the preparation of the first and second nucleotides, and nucleic acid amplification thereof, as recited in claim 8, as discussed previously, and advantages thereof.


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In view of the foregoing comments and amendments, reconsideration and allowance of all claims being considered on the merits in the above-identified application, are respectfully requested.

Applicants request any shortage of fees due in connection with the filing of this paper be charged to the Deposit Account of Antonelli, Terry, Stout & Kraus, LLP, Deposit Account No. 01-2135 (case 1021.43085X00), and credit any excess payment of fees to such Deposit Account.

Respectfully submitted,

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